

## Thermal stimulation of Ross®-lineage embryos on a commercial scale

### Estimulação térmica dos embriões da linhagem Ross® em escala comercial

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#### ABSTRACT

*Artificial incubation is an essential process to obtain healthy birds with good performance; nevertheless, it requires sustained improvement. During this process, incubation temperature is considered a critical factor, which has been studied. The objective of this study was to evaluate the development of Ross® embryos after hot and cold thermal stimulation. To this end, temperatures 1.39°C above the standard temperature and a temperature fixed at 36.00°C that varied 1.00 to 0.30°C below the standard temperature were applied during the final embryonic development period (days 14 to 18) for three hours, on a commercial scale. Results revealed that hot and cold thermal stimulations did not cause embryo mortality; the hatching and chick quality index were maintained and even increased. Therefore, we believe that thermal stimulation has the potential to improve hatchery index, and thus grange performance; however, adjustments are needed, varying according to each individual hatchery, before it can be used as a protocol.*

**Key words:** commercial incubation, embryonic development, hatching, thermal conditioning.

#### RESUMO

*A incubação artificial é um processo fundamental para obtenção de aves saudáveis e com bom rendimento, contudo, necessita de constantes melhorias. Nesse processo, a temperatura de incubação é considerada um fator crítico e vem sendo estudada. O objetivo deste trabalho foi avaliar o desenvolvimento de embriões da linhagem Ross® frente a estimulações térmicas por calor e por frio. Para tanto, temperaturas de 1,39°C acima da temperatura padrão e de 36,00°C fixas, abaixo da temperatura padrão (variando de 1,00 a 0,30°C), foram aplicadas no último período de desenvolvimento embrionário (14º ao 18º dia) por três*

*horas de duração, em escala comercial. Com esse experimento, percebeu-se que as estimulações térmicas por calor e por frio não causaram mortalidade embrionária, mantiveram e até aumentaram os índices de eclosão, bem como a qualidade do pintinho. Dessa forma, acredita-se que as estimulações térmicas têm potencial de melhorar os índices do incubatório, e consequentemente o desempenho na granja, contudo necessita de ajustes, que dependem de cada incubatório para ser utilizada como protocolo.*

**Palavras-chave:** condicionamento térmico, desenvolvimento embrionário, eclosão, incubação comercial.

#### INTRODUCTION

Cellular differentiation during incubation results in embryonic development of organs and physiological regulation systems, such as thermoregulatory system, with the developmental phases overlapping in a continuous process. Organs involved in thermoregulation (hypothalamus, thyroid, and pituitary gland) develop during growth phase, but their final maturation occurs in the last days of incubation and shortly after hatching (FLORES et al., 2013). Thus, incubation climate may influence embryonic development, and also affect post-hatching performance. In this context, slight variations in environment during embryonic development, in particular temperature variations, are believed to

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induce epigenetic adaptations (GILBERTS & EPEL, 2009) that are important for bird adaptability.

Different temperatures during incubation may have different effects on broiler weight after hatching, and may affect the final slaughter weight (TONA et al., 2004; WILLEMSSEN et al., 2008); these variations may also increase tolerance to environmental temperature challenges (MORAES et al., 2003; COLLIN et al., 2007), alter post-natal growth (COLLIN et al., 2005; HALEVY et al., 2006), and resulted in improved performance on site at 38 days of age (SHINDER et al., 2009). These long-term adaptations occur after application of periodic thermal manipulation during the last phase of maturation, when embryos are most responsive to “training” (TZSCHENTKE & HALLE, 2009). Thus, the objective of this study was to evaluate the development of Ross® embryos on a commercial scale, by assessing hatchery productivity index and chick quality after subjecting them to two thermal stimulations (hot and cold) during the final stage of embryonic development (days 14 and 18) for a period of three hours each.

## MATERIALS AND METHODS

Eggs from the same batch of Ross® breeder hens between 61 and 63 weeks of age, were subjected to temperature variations during embryonic development in a modular single-stage incubator (SmartPro 77; Pas Reform Hatchery Technology, 2014, Zeddum, The Netherlands), which was used to combine different treatments. The tests were applied on a commercial scale, with the machines loaded at maximum capacity (76.800 eggs) using the hatchery incubation program as a baseline. T1 was 1.39°C above the 36.55°C programmed value for the 14<sup>th</sup> day of embryonic development (ED), and this was increased by 1.39°C for each subsequent programmed value until the 18<sup>th</sup> day (ED), for three hours per day; and T2 was a cold stimulus fixed at 36.00°C, varying 1.00°C to 0.30°C below the programmed temperature from the 14<sup>th</sup> to the 18<sup>th</sup> day of ED, for three hours per day. There were “control” incubations in the same single-stage machines (SS Cont), and some hatchings were also monitored in multiple-stage machines (CASP CM 125R, Amparo, Brazil) (MS Cont), both without thermal stimulation. The incubation environment (temperature, humidity, gas exchange, and ventilation) was monitored in real time using Smart Center software (Pas Reform Hatchery Technologies, 2014, Zeddum, The Netherlands).

Egg shell surface temperatures were recorded before and after the stimulations in 42

eggs distributed over three points in the incubator on pre-established and identified trays (as shown in Figure 1), using an infrared thermometer (ITR 4520, Braun Termoscan®, Kronberg, Germany) with an accuracy of  $\pm 0.20^{\circ}\text{C}$ . Thus, all trays in the upper, middle, and lower parts of the trolleys and the incubator were sampled. After hatching, an embryo diagnosis was performed to categorize losses in non-hatched eggs. Six trays with a capacity of 150 eggs each that had been monitored for temperature, were analyzed for each treatment.

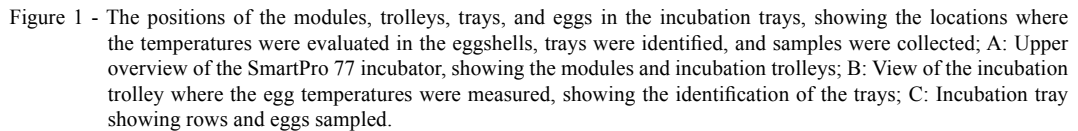
From these same trays, a random sample of 25 chicks (male and female) was evaluated for general quality using the Pasgar score (VAN DE VEN, 2011). Five items were verified: vitality (reflexes), navel, legs, beak, and abdomen. In addition, the cloacal temperatures of 15 males were measured using an infrared thermometer (ITR 4520). They were later sacrificed by cervical dislocation, in accordance with animal welfare standards. Next, the animals and their organs (yolk sac, heart, gastrointestinal tract [GIT], duodenal loop with pancreas, proventriculus, and ventriculus) were weighed using a scale (WeighMax® W- 3805 -100G, China) with a precision of  $\pm 0.01\text{g}$ .

In addition, at the end of incubation, the hatchability of the fertile eggs (total chicks hatched/total fertile eggs  $\times 100 = \%$ ), the total hatching rate (number of chicks/number of incubated eggs  $\times 100 = \%$ ), and the fertility (total fertile eggs/total incubated eggs  $\times 100 = \%$ ) were calculated. This study was performed in a random and observational manner in a factorial scheme ( $1 \times 2 \times 2$  – one age range of breeder hens  $\times$  two thermal treatments and two controls). The data were subjected to analysis of variance (ANOVA) through PROC GLM; later, averages were compared using Tukey tests with a 95% confidence level using SAS version 9.0 (2010).

## RESULTS AND DISCUSSION

The surface temperatures were higher among the chicks that had received thermal treatments than for those in the control groups (no stimulation) (Table 1). The weight of the heart was lower in the T1 group (hot stimulus: 1.39°C) than in other groups. This observation was consistent with the literature, in which overheated embryos showed reduced heart size and altered cardiac muscle development (LEKSRISOMPONG et al., 2007) with an eggshell temperature of 38.90°C.

The masses of the GIT and the duodenal loops with the pancreas were greater ( $P < 0.05$ ) in the single-stage incubated groups, both control and



Total weight of chicks and free weight of yolks did not differ significantly ( $P>0.05$ ) between groups (Table 1). Tendencies towards lower average total weight were observed in T1 (heat stimulation:  $1.39^{\circ}\text{C}$ ), followed by the SS control group (no stimulus - single stage) and the T2 group (cold

stimulation: 36.00°C). WINELAND et al. (2006ab) and LEKSRI SOMPONG et al. (2007) reported that lower average weights of chicks and their organs after heat stimulation. Other researchers (YALÇIN et al., 2008) increased the incubation temperature to 38.5°C for six hours per day from the 10<sup>th</sup> to the 18<sup>th</sup> day, and reported accelerated growth compared to the control group with greater chick weight. This effect was not observed by YALÇIN & SIEGEL (2003), who assessed the effects of higher temperature (39.00°C). Note that the difference is only 0.50°C, which essentially corresponds to 1.00°F, the unit normally used in incubation (FLORES et al., 2013). This emphasizes the narrowness of temperature variation, and that its effects are dependent on the period of ED, as well as the frequency and intensity of the thermal stimulation. Stimulation used in this study was at most 38.33°C for heat and 36.00°C for cold, in alternation; that is, the embryos received both

Table 1 - Average surface temperatures, weights, embryo diagnoses, and hatchery productivity in the thermal stimulation and control groups.

Analyses	Variable	Treat.	Average	Variable	Treat.	Average
Surface Temperature and Weight	Surface temperature (°C)	T1	37.99 <sup>a</sup> ±0.22	Weight of heart (g)	T2	0.41 <sup>a</sup> ±0.01
		T2	37.84 <sup>a</sup> ±0.22		SS Cont.	0.40 <sup>a</sup> ±0.01
		MS Cont.	37.47 <sup>ab</sup> ±0.38		MS Cont.	0.30 <sup>b</sup> ±0.02
		SS Cont.	37.13 <sup>b</sup> ±0.22		T1	0.30 <sup>b</sup> ±0.01
	Total weight (g)	MS Cont.	48.63 <sup>a</sup> ±1.76	Weight of GIT (g)	T4	2.12 <sup>a</sup> ±0.09
		T1	47.40 <sup>a</sup> ±1.02		SS Cont.	2.06 <sup>a</sup> ±0.09
		SS Cont.	47.01 <sup>a</sup> ±1.02		T1	1.99 <sup>a</sup> ±0.09
		T2	44.24 <sup>a</sup> ±1.02		MS Cont.	1.51 <sup>b</sup> ±0.15
	Free weight of yolk (g)	SS Cont.	41.82 <sup>a</sup> ±0.89	Weight of duodenal loop and pancreas (g)	T2	0.54 <sup>a</sup> ±0.02
		MS Cont.	40.86 <sup>a</sup> ±1.54		SS Cont.	0.54 <sup>a</sup> ±0.02
T1		40.81 <sup>b</sup> ±0.89	T1		0.50 <sup>a</sup> ±0.02	
T2		39.14 <sup>b</sup> ±0.89	MS Cont.		0.31 <sup>b</sup> ±0.04	
Weight of yolk (g)	MS Cont.	7.77 <sup>a</sup> ±0.57	Weight of PVV (g)	T2	3.03 <sup>a</sup> ±0.08	
	T1	6.58 <sup>ab</sup> ±0.33		SS Cont.	2.93 <sup>a</sup> ±0.08	
	SS Cont.	5.37 <sup>b</sup> ±0.33		T1	2.47 <sup>b</sup> ±0.08	
	T2	5.05 <sup>b</sup> ±0.33		MS Cont.	2.45 <sup>b</sup> ±0.14	
Embryo Diagnosis	Infertility (%)	T1	12.3	Pecked dead (%)	T1	0.4
		SS Cont.	15.6		SS Cont.	0.1
		T2	14.2		T2	0
	Mort. 1-4 days (%)	T1	5.6	Rotten (%)	T1	0.5
		SS Cont.	5.2		SS Cont.	2.1
		T2	9.4		T2	0.7
	Mort. 5-14 days (%)	T1	1.3	Cracked (%)	T1	0
		SS Cont.	0.6		SS Cont.	0.4
		T2	1.8		T2	1
	Mort. 5-14 days (%)	T1	1.3	Non-pecked live (%)	T1	0.1
SS Cont.		0.6	SS Cont.		0.3	
T2		1.8	T2		0	
Mort. 15-21 days (%)	T1	2.9	Fungus (%)	T1	0.1	
	SS Cont.	3.9		SS Cont.	0	
	T2	2.6		T2	0	
Incubation Index	Pecked live (%)	T1	0.6	Total eggs not born/not evaluated	T1	214/900
		SS Cont.	0.4		SS Cont.	258/900
		T2	0.6		T2	272/900
	General hatching (%)	T1	72.95	Fertility (%)	T2	87.7
		SS Cont.	67.54		SS Cont.	84.39
		MS Cont.	66.56		MS Cont.	-
		T2	67.27		T2	85.79
	Waste (%)	T1	1.42	Pasgar score (%)	T1	8.8
		SS Cont.	1.74		SS Cont.	8.8
		MS Cont.	2.74		MS Cont.	-
		T2	2.29		T2	9.08
	Hatchability (%)	T1	83.18			
		SS Cont.	80.03			
		T2	78.41			

T1 = Hot stimulus 1.39°C above programmed temperature for ED days 14 to 18/3 hours per day; T2 = Cold stimulus fixed at 36°C varying from an additional 1.0 to 0.3°C below programmed value for ED days 14 to 18/3 hours per day; S Cont. = Single stage; MS Cont. = Multiple stage; GIT = gastrointestinal tract; PVV = proventriculus and ventriculus; Treatments with different letters have statistical significance ( $P<0.05$ ) by Tukey test.

higher and lower temperature shocks. We were able to observe that the resulting alterations in embryonic development in this study did not compromise chick hatching rates or their quality.

The average yolk weight in the MS group (no stimulus - multiple stage) was significantly different from those of the other groups ( $P < 0.05$ ). According to the literature, increased residual yolk is a sign of overheating (LEKSRIOMPONG et al., 2007), which reaffirms the possibility that non-scheduled variations at inadequate times may negatively affect normal development. In the industry, temperature variability in different regions of the machine is often observed (FRENCH, 2002), since there are eggs of various origins and in different phases of development, in addition to differences in metabolism, oxygen consumption, and size due to breeder hens of different ages (LOURENS et al., 2006; HAMIDU et al., 2007). This study evaluated the development of embryos from Ross® breeder hens aged between 61 and 63 weeks. Thermal stimulation of other eggs from different age ranges of breeder hens is currently being evaluated as a continuation of the current study.

The residual yolk weights were 15.97%, 13.87%, 11.41%, and 11.42% for the control EM (no stimulation - multiple stage), T1 (hot stimulus: 1.39°C), SS control (no stimulation - single stage), and T2 (cold stimulus - fixed at 36.00°C) groups, respectively. Values lower than 10% of the chick's weight are recommended for good physical development (MEIJERHOF, 2005).

The chick overall quality can be used to identify possible incubation problems. Pasgar scores of at least nine points have been suggested to indicate non-problematic incubation (PASREFORM, 2010ab). The T1 (hot stimulus: 1.39°C) and the SS control (no stimulation - single stage) treatments scored 8.8 points, while the T2 group (cold stimulus - fixed at 36.00°C) scored 9.1 points.

Incorrect incubation temperatures may reduce hatchability, chick quality, and particularly affect post-hatching performance (HULET et al., 2007). In this study, the hatching rate of the T1 eggs (hot stimulus - 1.39°C) was greater than expected (Table 1), while the rates in the other groups did not differ significantly from the standard hatchery percentages. Previous studies have reported that thermal manipulation during the last development stage improves hatching by 1.5% and growth of males by 2.9%, with better food conversion (TZCHENTKE & HALLE 2009).

Evaluation of the non-hatched eggs (Table 1) revealed no indications of late embryonic

mortality that could be attributed to the high incubation temperatures, nor were significant percentages of non-pecked eggs found, which could be attributed to low temperatures. Average embryo temperatures were monitored and it did not exceed the standard limit. In addition, after the stimulation, the eggs would return to normal temperature within three hours.

LOURENS et al. (2005) established a target temperature of approximately 37.78°C for broiler embryos from the first day of incubation until transfer. Technical recommendation for single-stage incubations is 38.33°C in the last three days of incubation before transfer (maximum 38.61°C) (PAS REFORM, 2010ab) - that is, an increased embryonic temperature is recommended at the end of the cycle.

The exposure of eggs to high temperatures (38.50°C) for 4-6 hours between the 10<sup>th</sup> and 16<sup>th</sup> days of incubation may improve the capacity to adapt to heat stress in the fifth week (AKSIT et al., 2010). YALÇIN et al. (2010) reported better adaptation to high temperatures in broilers between the third and sixth post-hatching weeks, minimizing the negative effects caused by heat stress on slaughter weight and breast yield after exposure to temperatures of 39.60°C for 6h from the tenth to the eighteenth days of incubation. This study assessed the effects of temperatures 1.39°C above the scheduled values for each day of ED and with a temperature below these values (36.00°C), both for a period of three hours, from the 14<sup>th</sup> to the 18<sup>th</sup> days of ED. Results of this study showed no increase in embryonic mortality and improved hatching and quality index, suggesting the potential for improved farm performance.

However, depending on how the eggs are incubated, broilers reared in sheds may respond positively or negatively to temperature variations (LEKSRIOMPONG et al., 2009). For this reason, field data should be evaluated together with incubation data, and these batches should be monitored until slaughter. Considering that incubation conditions affect embryonic development, changes in broiler performance and health are to be expected (HULET et al., 2007; OVIEDO-RONDÓN et al., 2009). These effects may be observed in the batch averages or in small groups that experienced harmful microenvironments in the incubator. The current challenge is in reaching a consensus and defining protocols at industrial level that may improve productivity in the poultry sector (BOERJAN, 2010). In the meantime, the effects of sub-optimal incubation may cause problems in viability and poor health.

## CONCLUSION

Thermal stimulation administered for three hours from ED days 14 to 18, with temperatures 1.39°C above the standard temperature and at 36.00°C (below the standard), did not cause negative effects. Thus, thermal stimulation may be a potential tool for use in hatchery protocols; however, optimal temperatures should be determined based on the type of incubator.

## BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study was performed in accordance with the ethical principles of animal testing adopted by the Sociedade Brasileira de Ciência em Animais de Laboratório (SBCAL), and with the current legislation (Law 11,794 from 10/08/2008 and Decree 6,899 from 07/15/2009) under protocol number 3503-1, approved by the UNICAMP Ethics and Animal Welfare Committee.

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